NOTES ON THE EFFECTS OF THERAPEUTIC AGENTS ON TRYPANOSOMES IN RESPECT TO (*a*) ACQUIRED RESISTANCE OF THE PARASITES TO THE DRUG, AND (*b*) CHANGES IN VIRULENCE OF THE STRAINS AFTER ESCAPE FROM THE DRUG

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A. DEVELOPMENT OF DRUG-PROOF STRAINS

It is a well-established fact in the therapeutics of trypanosomiasis that, with each fresh recurrence of the parasites in any case, these become more resistant to the drug employed, and more difficult to drive out of the peripheral circulation.

This is not only the fact in any given case, but also as was first shown by Ehrlich[*] in the case of rats treated by atoxyl; the parasite itself, apart from the individual host, becomes resistant to the drug, and, when passed to fresh animals of the same species, reaches finally a condition in which it is resistant or 'fast' to the particular drug. This condition Ehrlich expressed by the term 'Atoxyl-fest' in the case of trypanosomes which had become refractory to the action of atoxyl.

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[*] Berl. Klin. Wochensch., 1907, p. 33.

We have observed similar effects, and they have also been noted by Plimmer and Thomson* in the case of atoxyl. Ehrlich† also found a developed resistance in the case of certain of the anilin colours used by him as trypanocides, the colours experimented with being para-fuchsin, trypan-red and trypan-blue. The special point which we believe is shown in regard to this resistance by the experiments here recorded is that it only holds for the species of animal in which it has been established, and disappears when the fast strain of trypanosomes is passed into another species of animal.

T. brucei was used in the experiments, and two distinct strains were used: (*a*) a strain which was atoxyl-fast in mice, for which we are indebted to the kindness of Prof. Ehrlich, and (*b*) an atoxyl-proof strain in the rat, obtained by ourselves after eight relapses. The results with the first of these strains are given in Table I, those with the second strain in Table II.

TABLE I

Strain atoxyl-fast in mouse as shown by first three experiments, but not fast in rat and dog as shown by subsequent experiments.

Experiment	Treatment	Duration of life	Examinations
1. Mouse (84)	0·5 c.c. of 2 per cent. acetylated atoxyl‡ repeated three times	8 days	Trypanosomes seen all the time, apparently diminished after second injection
2. Mouse (85)	As above	7·5 days	As above
3. Mouse (92)	,,	6 days	,,
4. Rat (265)	0·5 c.c. of 5 per cent. solution of atoxyl	19 days	Trypanosomes disappeared and reappeared in twenty days
5. Rat (266)	As above	24 days	,,
6. Dog (312)	10 c.c. of 10 per cent. solution of acetylated atoxyl in two injections	29 days	Trypanosomes disappeared and reappeared on twenty-sixth day

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* Proc. Roy. Soc., Vol. 79B, 1907, p. 504.

† *Loc. cit.*

‡ A supply of this substance was kindly sent us by Professor Ehrlich.

TABLE II

Strain atoxyl-fast in the rat as shown by first three experiments, but amenable to atoxyl in the mouse and dog as shown by the other experiments.

Experiment	Treatment	Duration of life	Examinations
1. Rat (289)	1 c.c. of 5 per cent. atoxyl	9 days	Trypanosomes were found on all but one day
2. Rat (292)	As above	11 days	Trypanosomes present all the time
3. Rat (298)	1 c.c. of 5 per cent. atoxyl, twice repeated	10 days	Trypanosomes present all the time
4. Mouse (94)	0·5 c.c. of 2 per cent. acetylated atoxyl given three times	23 days	Trypanosomes disappeared in twenty days
5. Mouse (97)	As above	26 days	Trypanosomes disappeared in twenty-three days
6. Dog (316)	10 c.c. of 10 per cent. acetylated atoxyl	19 days	Trypanosomes disappeared and reappeared in fourteen days

B. VARIATIONS IN VIRULENCE IN FRESH ANIMALS OF STRAINS WHICH HAVE REAPPEARED AFTER TREATMENT WITH DIFFERENT DRUGS

In our work on the treatment of Ngana (*T. brucei*) by atoxyl and mercury salts in rats, we have observed that when the parasites had reappeared after atoxyl treatment, and were then passed on into a fresh animal, they had acquired increased virulence in the process and caused death more rapidly than when passed on from animal to animal without any treatment. Accordingly a series of experiments with animals infected with *T. brucei*, *T. gambiense* and *T. dimorphon* was instituted specially to make observations on this point. In the case of the infections with *T. brucei* it was found that the virulence increased after attack with atoxyl, but was diminished after trypan-red. A slight increase in virulence was seen in the few experiments done with *T. dimorphon*, and a slight decrease in the case of *T. gambiense*, but the number of experiments and alteration in the virulence are both too small to warrant a definite conclusion in the case of these two species. The number of experiments with *T. brucei* is larger, and we are of opinion that this parasite after escape from atoxyl is, in the majority at least of the cases, more virulent than before treatment.

Previous experiments, without treatment with any drug, gave as the average duration of life after appearance of infection in the case of each strain as follows:—

T. brucei	6·3 days	Average of 146 rats
T. dimorphon	17·6 ,,	,, 56 ,,
T. gambiense	28·7 ,,	,, 36 ,,

No sudden alteration in virulence was found in the case of any of the strains. This had been specially watched for in the case of the strain of *T. brucei*, which had been under observation from 4th November, 1906, till 16th July, 1907; as stated, no increase of virulence was seen in the strain passed in the usual way from animal to animal without coming under the influence of any drug.

In order to infect the animals, an injection subcutaneously of 0·4 c.c. of infected blood, containing 6 to 8 parasites to the field (Zeiss, ocular 4, objective DD), was given in each case.

The results are shown in the following tables, which show the day of reappearance of trypanosomes in the peripheral circulation after their disappearance under the influence of the drug mentioned in each case, the number of animals then infected by the recurrent strain from the drug, the duration of life under this strain, and the increase in virulence marked +, or decrease marked — .

TABLE III

Virulence of *T. brucei* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(Untreated rats 146)	(6·3)	
23rd day	4 rats	4·6	+ 1·7
29th ,,	7 ,,	5·0	+ 1·3
19th ,,	2 ,,	6·0	+ 0·3
24th ,,	4 ,,	3·8	+ 2·5
26th ,,	3 ,,	6·8	− 0·5
38th ,,	7 ,,	4·2	+ 2·1
25th ,,	2 ,,	5·0	+ 1·3
19th ,,	3 ,,	6·0	+ 0·3
29th ,,	5 ,,	7·5	− 1·2
21s ,,	8 ,,	4·5	+ 1·8
35th ,,	6 ,,	4·0	+ 2·3
31st ,,	3 ,,	6·8	− 0·5

Taking the averages of the whole numbers of untreated and treated rats, we have :—

Untreated rats	146	Average duration of life	6·3 days
Post-atoxyl-treatment rats	54	,, ,, ,,	5·1 ,,
		Mean increase of virulence	1·2 ,,

The same strain of *T. brucei* treated with trypan-red in three experiments in which 11 rats were infected from recurrences after this drug, showed on the other hand a considerable decrease in virulence of the strain, as shown in the following table.

TABLE IV

Virulence of *T. brucei* recurrent after trypan-red treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(146 rats untreated)	(6·3)	
7th day	4 rats	15	− 9·7
11th ,,	3 ,,	9	− 2·7
5th ,,	4 ,,	11	− 4·7

Untreated rats	146	Average duration of life	6·3 days
Post-trypan-red-treatment rats	11	,, ,, ,,	11·9 ,,
		Mean decrease of virulence	5·6 ,,

The strain of *T. dimorphon*, after treatment with atoxyl, gave the following results in five experiments with 18 rats:—

TABLE V.

Virulence of *T. dimorphon* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(Untreated rats 56)	(17·6)	
29th day	5 rats	14·5	+ 3·1
42nd „	2 „	19·0	− 1·4
34th „	4 „	11·0	+ 6·6
28th „	3 „	19·0	− 1·4
26th „	4 „	16·0	+ 1·6

Untreated rats	56	Average duration of life	17·6 days
Post-atoxyl-treatment rats	18	„ „ „	15·3 „
		Mean increase in virulence	2·3 „

In four experiments with the strain of *T gambiense*, in which 20 rats were used, the following decrease in virulence was observed, but as stated above a much larger number of experiments would be required in order to definitely decide the point, for this much slower species of trypanosome:—

TABLE VI

Virulence of *T. gambiense* recurrent after atoxyl treatment.

Day of relapse	No. of animals infected with recurrent strain	Average duration of life in days	Change of virulence
	(36 rats untreated)	(28·7)	
59th day	6 rats	32	− 3·3
62nd „	7 „	30	− 1·3
54th „	4 „	39	− 10·3
68th „	3 „	31	2·3

Untreated rats	36	Average duration of life	28·7 days
Post-atoxyl-treatment rats	20	„ „ „	32·5 „
		Mean decrease in virulence	3·8 „

It may, perhaps, for clearness be emphasized that none of the animals mentioned in the Tables (III to VIII) received any treatment whatever. The comparisons are entirely of the virulence of the strains in what might be described as their natural condition, and when they have reappeared after a drug and are passed into a fresh host of the same animal where they are not further treated.